3. The Development of the Saprophytic Fungal Flora as Leaves Senesce and Fall

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Of the multitude of fungal spores of a vast array of species which are impacted on to leaf surfaces only relatively few succeed in colonizing the leaves as they senesce and grow as active saprophytes within the leaf tissues after death. Those that do can be grouped into those that are almost ubiquitous, the common primary saprophytes, and those which are more restricted in their host range, the restricted primary saprophytes (Hudson, 1968).

The fungi forming the basis of the first group are: *Cladosporium* spp., especially *C. herbarum* and *C. cladosporioides*; *Alternaria* spp., especially *A. tenuis*; *Aureobasidium pullulans*, *Epicoccum nigrum* and *Botrytis cinerea*. On most leaves which have been examined all these are present and exceptions are difficult to find. Kendrick and Burges (1962), for instance, only found *A. pullulans* on needles of *Pinus*, whereas on the moss *Pseudoscleropodium purum*, Kilbertus (1968) recorded *C. herbarum*, *A. tenuis* and *E. nigrum* but not *A. pullulans* and *B. cinerea*. In the tropics, *Alternaria* spp. are less common and are replaced by *Nigrospora* spp., especially *N. sphaerica*, and *Curvularia* spp., especially *C. lunata*, as is evident from examining senescent leaves of monocotyledons such as *Arundo donax*, *Panicum maximum* and *Musa sapientum* (Hudson, 1962; Meredith, 1962). These differences are also reflected in a comparison of the dry air-spora of tropical and temperate regions (Cammack, 1955; Hirst, 1953; Meredith, 1961; Richards, 1956). Westerdijk (1949) noted the association of these fungi on other substrata. They occur on cereal stubble with other fungi such as *Stemphylium* spp. and on weathered cotton fabrics. Christensen and Kaufmann (1965), in their studies on the deterioration of grain, designated these and others, such as *Fusarium*, *Chaetomium* and *Rhizopus* spp., as "field fungi" on weathered grain. This group of fungi is thus by no means confined to senescent and dead leaves but is almost always associated with freshly decaying green parts of plants.

The restricted primary saprophytes are much more variable and are confined to a particular host genus or a related group of plants. For example, *Readeriella mirabilis* and *Piggotia stellata* appear restricted to *Eucalyptus* (Macauley and Thrower, 1966) and *Ascochytula obionis* to *Halimione portulacoides* (Dickinson, 1965). Several species of *Leptosphaeria*, such as *L. microscopica*, are restricted to Gramineae and *Fusicoccum bacillare* and *Sclerophoma pithyophila* are both very common on needles of *Pinus* but the latter, at least, is also found on other coniferous leaves (Gremmen, 1959). In many of these substratum specificity might be synonymous with, and explained by, host specificity. Many of these, although very active saprophytes, may have an additional advantage in that they gain access as parasites. *S. pithyophila*, for instance, has been associated with the defoliation of the current year's needles of *Pinus sylvestris* (Batko *et al.*, 1958).

I want to consider what attributes the common primary saprophytes possess which enable them to be such widespread and successful colonizers of leaves and incidentally why the multitude of "casual inhabitants" of plant surfaces do not also colonize. The term "casual inhabitants" is used here in the sense of Dickinson (1967) to distinguish fungi active in the phylloplane and leaf-inhabiting saprophytes from these, which are impacted by chance on to leaf surfaces merely because they are wind dispersed. Several other contributors to this Symposium have considered or will consider some of these attributes.

From studies of the phylloplane it is clear that these fungi arrive at the leaf surface mainly as airborne conidia. Although they are the imperfect states of Ascomycetes, ascospores probably play little part in their dispersal. It is also clear that they only become vegetatively active within the leaf with the onset of senescence. With such fungi the distinction between weak parasitism and saprophytism may be difficult to make. As with the restricted primary saprophytes perhaps the ability to parasitize living tissue would obviously give them an initial advantage over strict saprophytes. It is well known that *Botrytis cinerea* can invade sound tissues, killing in advance of its hyphae and that it is a common facultative parasite. However, there is little direct evidence that it, or the others, can or do invade sound leaf tissues. Both *B. cinerea* and *A. pullulans* are usually considered as pectolytic fungi. The latter was recorded in significant frequency as a surface colonizer of pine needles by Kendrick and Burges (1962). Smit and Wieringa (1953) found it on deciduous tree leaves as soon as they unfolded and in the bud stage and Hudson and Webster (1958) frequently found it on leaves of *Agropyron repens* before they unfolded. From the results of these last two groups of workers, one is led to conclude that *A. pullulans* is either a systematic and symptomless parasite or that chance infiltration of conidia had

occurred from the phylloplane of other leaves. No necrotic lesions were recorded by any of these workers whereas Frankland (1966) did find that it caused superficial lesions on the stalks of *Pteridium*. Comparable lesions occur on linseed and flax (*Linum usitatissimum*) and are caused by *A. pullulans* var. *lini*. It is the causal organism of stem break and browning of these and other *Linum* spp. (Sanderson, 1965).

If thus regarded as saprophytes, it should be considered whether they are strictly "sugar fungi" which exploit the leaves as long as relatively ephemeral soluble carbon sources, such as hexoses and pentoses, or other carbon compounds simpler than cellulose, such as pectins, starch and hemicelluloses, are available. It should also be considered whether they possess the other physiological characteristics of such fungi, in particular, a high mycelial growth rate and a capacity for rapid spore germination (Garrett, 1956, 1970).

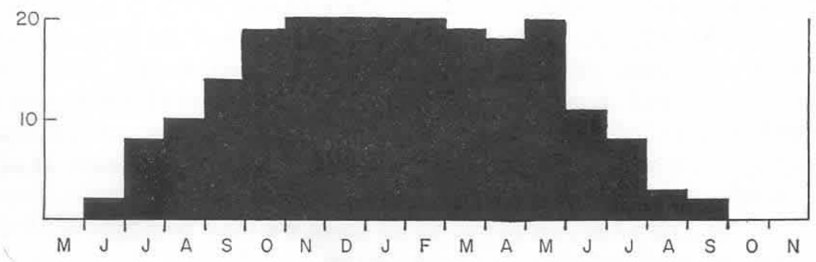

FIG. 1. Frequency of *Cladosporium herbarum* on monthly samples of 20 leaves of *Fagus sylvatica* (from Hogg and Hudson, 1966).

Their persistence on leaves, which may be illustrated by considering the distribution and frequency of *C. herbarum* on leaves of *Fagus sylvatica* (Hogg and Hudson, 1966), would suggest that they are not strictly confined to the more ephemeral carbon sources. In Fig. 1, the histograms express the presence or absence of sporulating conidiophores of *C. herbarum* on monthly samples of 20 leaves. The leaves were incubated for 2 days in a damp chamber after collection to induce sporulation. The fungus colonized and sporulated on damaged necrotic parts of the leaves in June, within 2 months of unfolding. By leaf fall, in October, it had already attained a high frequency and it persisted with only slight fluctuations until the following June and disappeared after September. The other common primary saprophytes appeared in lower frequency but had a similar distribution.

The ability to utilize cellulose is often regarded as essential for saprophytic fungi and the majority, except most Phycomycetes, can do this. Siu (1951) lists *C. herbarum*, *A. tenuis* and *E. nigrum* as having

definite cellulolytic activity but not *A. pullulans*. Reese and Levinson (1952) also found *A. pullulans* non-cellulolytic but reported cellulolytic activity of *B. cinerea* on cotton duck and also that it produces large amounts of pectinase. This was confirmed for *B. cinerea* by Hancock *et al.* (1964). On this evidence only *A. pullulans* appears non-cellulolytic. However, we must appreciate that different isolates of the same fungus may vary in their cellulolytic, and also pectolytic, activities. Hogg (1966) found considerable variation in the ability of three isolates of *A. tenuis* from *Fagus* leaves to utilize filter paper cellulose. Hering (1967) found no evidence for pectolytic activity in isolates of *A. pullulans* from *Quercus* leaves. He also inoculated leaves, sterilized by γ irradiation, separately with *A. pullulans*, *C. herbarum* and other leaf-and litter-inhabiting fungi. The dry weight loss caused by the former two fungi was extremely low (under 2% and 4% respectively) even after 6 months incubation at 9–15°C whereas, for comparison, *Mycena galopus*, an actively cellulolytic litter inhabitant, caused a loss of 15–20%. This loss corresponded with the utilization of about one sixth of the total cellulose present. However, on similarly treated *Pteridium* stalks, Frankland (1969) found that *A. pullulans* caused about a 7% loss of dry weight in 6 months and that there was some evidence that it decomposed a small quantity of cellulose and lignin as well as soluble carbohydrates. It also penetrated into the lignified xylem.

Although the situation is not at all clear we may conclude that the common primary saprophytes are by no means all strict "sugar fungi", although some strains of *A. pullulans*, and *C. herbarum*, may possess very low or no cellulolytic activity. However, we cannot argue that they use this cellulolytic ability in colonization. They presumably use simple carbohydrates as long as they last and then those that can, go on to degrade cellulose and thus persist. This may be placing an undue emphasis on their carbohydrate nutrition to the neglect of their nitrogen requirements. Here there is a gap in our knowledge. In culture they can all use nitrates, ammonia or amino acids as their sole nitrogen source but we know nothing of what is available to them within the leaf. Indications of the over-riding limitations of their nitrogen supply are seen when leaves are amended with an available source. Burchill and Cook (this Section, Chapter 6) will show that spraying apple leaves with 5% urea after harvest but before leaf-fall resulted in dense populations of *Alternaria* spp., and an increase in the concentration of *Cladosporium* spp., *Fusarium* spp., *Gonatobotrys simplex* and other saprophytes, and that immersing the leaves in 5% urea at leaf fall had the same effect, except that populations of *Alternaria* spp. remained low. I should like to illustrate this response to additional available nitrogen by considering some of my own results. Fallen apple leaves (cultivar Bramley's

seedling) were immersed in 5% urea in mid November, soon after leaf fall, drained and placed on the ground under "Netlon" covered frames under the tree. Leaves were examined at four time intervals up to mid January by which time the urea-treated leaves were fragmented and incorporated into the soil by earthworms. Two techniques were used. 10 treated and 10 untreated leaves were scored for presence of sporulating fungi after 2 days' incubation in a damp chamber. The most common fungi present were *C. herbarum*, *E. nigrum*, *A. tenuis* and *Phoma* sp. (Table I). *B. cinerea* was not recorded and the technique is unsuitable for the detection of *A. pullulans*. Treatment with urea maintained the high frequency of *C. herbarum*, increased the frequency, in terms of number of leaves colonized, of *E. nigrum* and *Phoma* sp. and

TABLE I. Colonization of urea treated and untreated apple leaves

Fungus	Treatment	weeks after treatment			
		1	3	7	9
Cladosporium herbarum	none	10	10	10	10
	urea	10	10	10	—
Epicoccum nigrum	none	0	4	5	3
	urea	0	10	9	—
Phoma sp.	none	0	5	8	9
	urea	1	8	10	—
Alternaria tenuis	none	0	3	3	1
	urea	0	0	0	—

decreased the frequency of *A. tenuis*. There was also a noticeable increase in *Mucor* and *Fusarium* spp. and *Gonatobotrys simplex* on urea treated leaves.

Discs, 1 cm in diameter, were also cut from the leaves and incubated for 1 day to induce sporulation before being vigorously shaken in sterile water for 1 h to remove spores. Dilution plates were made from the washing water. These yielded additional information about *C. herbarum*, *A. pullulans* and *Phoma* sp. The dilutions were too great to detect appreciable quantities of *A. tenuis* and *E. nigrum*. From the total viable spore count (Table II), it can be seen that there was a marked increase with the urea-treated leaves, but that the increase was less pronounced in the third week after treatment than it was after the first and seventh weeks. This is explicable in terms of the different effects, direct or indirect of the urea on the different fungi. There was some stimulation of *C. herbarum* by urea but there was a fall-off by the seventh week.

A. pullulans formed over 90% of the total spore population on both the untreated and urea-treated leaves after the first week, but it had increased over threefold on the treated leaves. By the third week its density fell markedly so that it formed only 8% of the total population as compared with over 40% of the untreated leaves. The build up of *Phoma* sp. occurred later in both. By the seventh week it accounted for over 80% of the total population on treated leaves as compared with only 33% on untreated leaves.

Obviously urea has different effects on different fungi and it could be that it is the available nitrogen which is one of the major determinants controlling colonization. For instance, it has been noted that *A. pullulans* is hardly, if at all, cellulolytic. The addition of a readily available nitrogen source may stimulate it to utilize more rapidly the

TABLE II. Colonies developing from leaf washings ($\times 10^3/cm^2$)

Fungus	Treatment	weeks after treatment			
		1	3	7	9
Total	none	66	97	75	54
	urea	219	129	226	—
Cladosporium herbarum	none	2	53	37	19
	urea	6	64	23	—
Aureobasidium pullulans	none	64	40	13	11
	urea	204	11	2	—
Phoma sp.	none	0	0	25	21
	urea	0	34	184	—

limited supply of simple carbohydrates present, after which it rapidly disappears, whereas the cellulolytic fungi persist and increase because of the increased nitrogen supply which enables them to utilize more cellulose.

Senescing and fallen leaves are very prone to desiccation and are also subject to strong sunlight. Thus other attributes worth considering are any mechanisms possessed by these common primary saprophytes, which enable them to tolerate desiccation and ultra-violet light. Both these environmental factors are considered in some detail by other contributors but I should like to consider some aspects associated with these here. Webster and Dix (1960) compared the growth rates of three of these, *C. herbarum*, *A. tenuis* and *E. nigrum* with those of two fungi *Torula herbarum* and *Tetraploa aristata*, which appear later in the succession on grasses. They found that there was little difference between the capacity

of the mycelium of the various colonizers to grow at low humidities and also that the former did not make better growth at low humidities. But under favourable humidities (100% RH), *A. tenuis* and *E. nigrum* not only grow faster than the secondary colonizers, but they also had a shorter latent period before germination and their germ tubes grew faster (Table III). They behaved as typical sugar fungi in these respects. These features, coupled with the fact that their conidia can germinate at lower relative humidities would give them an advantage over the others in that their conidia would germinate under less ideal conditions of humidity, and they would quickly exploit, by virtue of their more rapid growth rate, any change to more humid conditions. Diem has considered the survival at low humidities of germinating conidia of

TABLE III. Mycelial growth rate, latent period for germination, growth rate of germ tubes at 100% RH and lowest RH at which spores germinated (after Webster and Dix (1960)).

Primary Colonizers	Mycelial growth rate (mm/day)	Latent period (h)	Germ tube growth rate (μm/h)	Lowest RH at which germination occurs
Cladosporium herbarum	2·96	6–12	4·2	89%
Alternaria tenuis	6·41	3–6	29·1	89%
Epicoccum nigrum	6·45	0–3	31·6	92%
Secondary Colonizers				
Torula herbarum	1·41	12–18	0	Water
Tetraploa aristata	3·34	12–18	2·4	98%

C. herbarum, *A. tenuis* and some "casual inhabitants" of the phylloplane at low humidities. He concluded that the high sensitivity of hyaline germinated conidia would account for the paucity of *Penicillium* and *Aspergillus* spp. in the phylloplane, whereas the germinated conidia of *C. herbarum* were far more resistant to drying at 65% RH, and that its mycelium would be more suited to persist on the leaf surface (and, we might suggest, to colonize the leaf). The lack of any requirement for exogenous nutrients for germination by these fungi, as described by Stott (this volume), may also be considered as a beneficial attribute and relevant here.

Once established in the leaf tissues, most of these fungi rapidly produce some form of deeply pigmented survival structures. *C. herbarum* produces minute microsclerotia, *E. nigrum* and *B. cinerea* sclerotia and *A. pullulans* chlamydospores. *C. herbarum*, *E. nigrum* and *A. tenuis* also have pigmented conidia. In the two latter they are, in

addition, multicellular. All have a pigmented mycelium and some, such as *C. herbarum* and *A. pullulans*, produce chlamydospores in groups. Such features were listed by Nicot (1960) as being protective mechanisms against desiccation and strong sunlight. Pigmentation may certainly be associated with increased tolerance to ultra-violet light. Some aspects of this are discussed by Pugh and Buckley (this volume). The lethal effects of the ultraviolet component of sunlight have been observed on hyaline conidia (Weille, 1961; Dowding, 1969). Pigmented conidia appear to be resistant to such light. It is worth noting that the conidia of *B. cinerea* and initially those of *A. pullulans* are hyaline.

It is thus clear that the common primary saprophytes possess a multiplicity of attributes, some of which have been considered here, by which they have become adapted to their relatively inhospitable niche and that each fungus has its own particular complex of attributes, perhaps varying from any other, but whose summation produces an equally well-adapted fungus.

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